

## VI. CLAIMS

What is claimed is:

- 1 A method of practically producing an equine mammal comprising the steps of:
  - a. determining an estimated time of estrus of a female species of an equine
  - 5 mammal, said female species having two uterine horns, each uterine horns having a tip and a follicle, and having a vagina, a uterus, and a rectum;
  - b. collecting equine sperm cells from a male species of an equine mammal;
  - c. establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal;
  - 10 d. establishing a flexible probe having a sperm container;
  - e. placing said flexible probe in the vagina of said female equine mammal;
  - f. manipulating said flexible probe into said uterus of said female equine mammal;
  - g. guiding said flexible probe into a uterine horn of said female equine mammal;
  - 15 and
  - h. gently manipulating said flexible probe per rectum as it is guided deep within said uterine horn of said female equine mammal to a location deep within said uterine horn of said female species near the tip of said uterine horn;
  - i. artificially inseminating said female species of said equine mammal;
  - 20 j. fertilizing at least one equine egg within said female species of said equine mammal; and
  - k. producing an equine offspring mammal.
- 2 A method of practically producing an equine mammal as described in claim 1 wherein said step of establishing an equine insemination sample containing at least some of  
25 said equine sperm cells from said male species of said equine mammal comprises the steps of:
  - a. determining the sex characteristic of a plurality of said equine sperm cells; and
  - b. sorting said equine sperm cells according to the determination of their sex characteristic,

and wherein said step of producing an equine offspring mammal comprises the step of producing an equine offspring mammal of the desired sex.

3 A method of practically producing an equine mammal as described in claim 2 wherein  
said step of establishing an equine insemination sample containing at least some of  
5 said equine sperm cells from said male species of said equine mammal comprises the  
step of establishing an equine insemination sample containing at least some of said  
equine sperm cells from said male species of said equine mammal and having a low  
number of said equine sperm cells relative to the typical artificial insemination  
dosage.

10 4 A method of practically producing an equine mammal as described in claim 3 wherein  
said step of establishing an equine insemination sample containing at least some of  
said equine sperm cells from said male species of said equine mammal and having a  
low number of said equine sperm cells relative to the typical artificial insemination  
dosage comprises the step of establishing an equine insemination sample containing at  
15 least some of said equine sperm cells from said male species of said equine mammal  
and having a low number of said equine sperm cells relative to the typical artificial  
insemination dosage selected from the group consisting of: an equine insemination  
sample of no more than about five million sperm cells, and an equine insemination  
sample of no more than about twenty-five million sperm cells.

20 5 A method of practically producing an equine mammal as described in claim 4 wherein  
said step of fertilizing at least one equine egg within said female species of said  
equine mammal comprises the step of fertilizing at least one equine egg within said  
female species of said equine mammal at success levels statistically comparable to the  
typical artificial insemination dosage.

25 6 A method of practically producing an equine mammal as described in claim 5 wherein  
said step of establishing an equine insemination sample containing at least some of  
said equine sperm cells from said male species of said equine mammal and having a

low number of said equine sperm cells relative to the typical artificial insemination dosage comprises the steps of:

- a. staining said equine sperm cells;
- b. sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry; and
- c. concentrating said sorted equine sperm cells.

7 A method of practically producing an equine mammal as described in claim 6 wherein said step of sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry comprises the step of collecting live sperm of the desired sex at the rate of at least nine hundred live sperm per second.

8 A method of practically producing an equine mammal as described in claim 6 wherein said step of sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry comprises the step of operating a high speed cell sorter at a pressure of at least about fifty pounds per square inch.

9 A method of practically producing an equine mammal as described in claim 1 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal and having a low number of said equine sperm cells relative to the typical artificial insemination dosage.

10 A method of practically producing an equine mammal as described in claim 9 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal and having a low number of said equine sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal

and having a low number of said equine sperm cells relative to the typical artificial insemination dosage selected from the group consisting of: an equine insemination sample of no more than about five million sperm cells, and an equine insemination sample of no more than about twenty-five million sperm cells.

- 5      11      A method of practically producing an equine mammal as described in claim 4 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample having a volume selected from the group: 0.2 ml or 1ml.
- 10      12      A method of practically producing an equine mammal as described in claim 7 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample having a volume selected from the group: 0.2 ml, or 1ml.
- 15      13      A method of practically producing an equine mammal as described in claim 10 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample having a volume selected from the group: 0.2 ml, or 1ml.
- 20      14      An animal produced through use of a method as described in any of claims 1, 4, 7, 11, or 12.
- 15      A method of practically producing an equine mammal comprising the steps of:
- 25      a.      determining an estimated time of estrus of a female species of an equine mammal, said female species having two uterine horns, each uterine horns having a tip and a follicle;
- b.      collecting equine sperm cells from a male species of an equine mammal;

- c. establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal;
- d. inserting at least a portion of said equine insemination sample deep within at least one of said uterine horns of said female species near the tip of said uterine horn;
- e. artificially inseminating said female species of said equine mammal;
- f. fertilizing at least one equine egg within said female species of said equine mammal; and
- g. producing an equine offspring mammal.

5

10      16      A method of practically producing an equine mammal as described in claim 15 and further comprising the step of ascertaining which uterine horn is ipsilateral to the preovulatory follicle and wherein said step of inserting at least a portion of said equine insemination sample deep within at least one of said uterine horns of said female equine species comprises the step of inserting at least a portion of said equine insemination sample near the tip of said uterine horn ipsilateral to the preovulatory follicle.

15

17      A method of practically producing an equine mammal as described in claim 15 wherein said step of artificially inseminating said female species of said equine mammal comprises the step of artificially inseminating said female equine species on a single occasion close to ovulation.

20

18      A method of practically producing an equine mammal as described in claim 17 wherein said step of artificially inseminating said female equine species on a single occasion close to ovulation comprises the step of comprises the step of artificially inseminating said female equine species both ipsi- and contra-lateral within the uterine horns of said equine mammal.

25

19      A method of practically producing an equine mammal as described in claim 15 wherein said steps of inserting at least a portion of said equine insemination sample

deep within at least one of said uterine horns of said female species near the tip of said uterine horn, artificially inseminating said female species of said equine mammal, and fertilizing at least one equine egg within said female species of said equine mammal are each accomplished in a field environment.

- 5      20      A method of practically producing an equine mammal as described in claim 15 wherein said step of inserting at least a portion of said equine insemination sample deep within at least one of said uterine horns of said female species near the tip of said uterine horn comprises the step of inserting at least a portion of said equine insemination sample at a location selected from the group consisting of: within about  
10      one-half inch of the tip of said uterine horn, within about one inch of the tip of said uterine horn, and within about two inches of the tip of said uterine horn.
- 21      A method of practically producing an equine mammal as described in claim 15 and further comprising the step of manipulating the ovulation of said female equine mammal prior to accomplishing the step of artificially inseminating said female  
15      species of said equine mammal.
- 22      A method of practically producing an equine mammal as described in claim 21 wherein said step of manipulating the ovulation of said female equine mammal prior to accomplishing the step of artificially inseminating said female species of said equine mammal comprises the step of administering a gonadotropin releasing  
20      hormone to said female equine mammal.
- 23      A method of practically producing an equine mammal as described in claim 22 wherein said step of artificially inseminating said female species of said equine mammal is accomplished at a time selected from the group consisting of: about thirty  
25      four hours after said step of administering said gonadotropin releasing hormone to said female equine mammal, about forty hours after said step of administering said gonadotropin releasing hormone to said female equine mammal, and between about

thirty four hours to about forty hours after said step of administering said gonadotropin releasing hormone to said female equine mammal.

24 A method of practically producing an equine mammal as described in claim 21  
wherein said step of inserting at least a portion of said equine insemination sample  
5 deep within at least one of said uterine horns of said female species near the tip of said  
uterine horn comprises the step of inserting said equine insemination sample within  
said uterine horn through the use of a flexible plastic artificial insemination pipette.

25 A method of practically producing an equine mammal as described in claim 15  
wherein said step of inserting at least a portion of said equine insemination sample  
10 deep within at least one of said uterine horns of said female species near the tip of said  
uterine horn comprises the steps of:

- a. establishing a flexible probe having a sperm container;
- b. placing said flexible probe in the vagina of said female equine mammal;
- c. manipulating said flexible probe into said uterus of said female equine  
15 mammal;
- d. slowly guiding said flexible probe into a uterine horn of said female equine  
mammal; and
- e. gently manipulating said flexible probe per rectum as it is guided deep within  
said uterine horn of said female equine mammal to a location deep within said  
20 uterine horn of said female species near the tip of said uterine horn.

26 A method of practically producing an equine mammal as described in claim 15 and  
further comprising the step of confirming by transrectal ultrasonography the location  
at which said step of inserting at least a portion of said equine insemination sample is  
accomplished prior to accomplishing said step of artificially inseminating said female  
25 species of said equine mammal.

27 A method of practically producing an equine mammal as described in claim 15  
wherein said step of establishing an equine insemination sample containing at least

some of said sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample having a low number of said equine sperm cells relative to the typical artificial insemination dosage, and wherein said step of fertilizing at least one equine egg within said female species of said equine mammal comprises the step of fertilizing at least one equine egg within said female species of said equine mammal at success levels statistically comparable to the typical artificial insemination dosage.

28 A method of practically producing an equine mammal as described in claim 27 wherein said step of establishing an equine insemination sample having a low number of said equine sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an equine insemination sample selected from the group consisting of: an equine insemination sample of no more than about five million sperm cells, and an equine insemination sample of no more than about twenty-five million sperm cells.

29 A method of practically producing an equine mammal as described in claim 25 wherein said step of establishing an equine insemination sample containing at least some of said sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample having a low number of said equine sperm cells relative to the typical artificial insemination dosage, and wherein said step of fertilizing at least one equine egg within said female species of said equine mammal comprises the step of fertilizing at least one equine egg within said female species of said equine mammal at success levels statistically comparable to the typical artificial insemination dosage.

30 A method of practically producing an equine mammal as described in claim 29 wherein said step of establishing an equine insemination sample having a low number of said equine sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an equine insemination sample selected from the group consisting of: an equine insemination sample of no more than about five million



sperm cells, and an equine insemination sample of no more than about twenty-five million sperm cells.

31 A method of practically producing an equine mammal as described in claim 29  
wherein said step of establishing an equine insemination sample containing at least  
5 some of said equine sperm cells from said male species of said equine mammal  
comprises the steps of:

- a. determining the sex characteristic of a plurality of said equine sperm cells; and
- b. sorting said equine sperm cells according to the determination of their sex  
characteristic,

10 and wherein said step of producing an equine offspring mammal comprises the step of  
producing an equine offspring mammal of the desired sex.

32 A method of practically producing an equine mammal as described in claim 31  
wherein said steps of determining the sex characteristic of a plurality of said equine  
sperm cells and sorting said equine sperm cells according to the determination of their  
15 sex characteristic comprise the steps of:

- a. staining said equine sperm cells;
- b. sorting according to said sex of said equine sperm cells through the use of high  
speed flow cytometry; and
- c. concentrating said sorted equine sperm cells.

20 33 A method of practically producing an equine mammal as described in claim 32  
wherein said step of sorting according to said sex of said equine sperm cells through  
the use of high speed flow cytometry comprises the step of collecting live sperm of  
the desired sex at the rate of at least nine hundred live sperm per second.

25 34 A method of practically producing an equine mammal as described in claim 32  
wherein said step of sorting according to said sex of said equine sperm cells through  
the use of high speed flow cytometry comprises the step of operating a high speed cell  
sorter at a pressure of at least about fifty pounds per square inch.

- 35      35      A method of practically producing an equine mammal as described in claim 32 wherein said step of sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry comprises the step of collecting equine sperm cells having the desired sex characteristic in a skim milk solution.
- 5      36      A method of practically producing an equine mammal as described in claim 33 wherein said step of establishing an equine insemination sample containing at least some of said sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample having a low number of said equine sperm cells relative to the typical artificial insemination dosage, and wherein  
10      said step of fertilizing at least one equine egg within said female species of said equine mammal comprises the step of fertilizing at least one equine egg within said female species of said equine mammal at success levels statistically comparable to the typical artificial insemination dosage.
- 15      37      A method of practically producing an equine mammal as described in claim 36 wherein said step of establishing an equine insemination sample having a low number of said equine sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an equine insemination sample selected from the group consisting of: an equine insemination sample of no more than about five million sperm cells, and an equine insemination sample of no more than about twenty-five  
20      million sperm cells.
- 25      38      A method of practically producing an equine mammal as described in claim 15 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the steps of:  
a.      determining the sex characteristic of a plurality of said equine sperm cells; and  
b.      sorting said equine sperm cells according to the determination of their sex characteristic,

and wherein said step of producing an equine offspring mammal comprises the step of producing an equine offspring mammal of the desired sex.

- 39 A method of practically producing an equine mammal as described in claim 25  
wherein said step of establishing an equine insemination sample containing at least  
5 some of said equine sperm cells from said male species of said equine mammal  
comprises the steps of:
- a. determining the sex characteristic of a plurality of said equine sperm cells; and
  - b. sorting said equine sperm cells according to the determination of their sex  
characteristic,

10 and wherein said step of producing an equine offspring mammal comprises the step of  
producing an equine offspring mammal of the desired sex.

- 40 A method of practically producing an equine mammal as described in claim 15 and  
further comprising the step of administering an equine pituitary extract to said to said  
female equine mammal to enhance the probability at which said step of fertilizing at  
15 least one equine egg within said female species of said equine mammal occurs.

- 41 A method of practically producing an equine mammal as described in claim 28  
wherein said step of establishing an equine insemination sample containing at least  
some of said equine sperm cells from said male species of said equine mammal  
comprises the step of establishing an equine insemination sample having a volume  
20 selected from the group: 0.2 ml, or 1ml.

- 42 A method of practically producing an equine mammal as described in claim 30  
wherein said step of establishing an equine insemination sample containing at least  
some of said equine sperm cells from said male species of said equine mammal  
comprises the step of establishing an equine insemination sample having a volume  
25 selected from the group: 0.2 ml, or 1ml.

- 43 A method of practically producing an equine mammal as described in claim 37 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample having a volume  
5 selected from the group: 0.2 ml, or 1ml.
- 44 An animal produced through use of a method as described in any of claims 15, 19, 23, 25, 28, 30, 31, 33, 35, 40, 41, , or 43.
- 45 A method of sorting equine sperm cells according to a determination of their sex characteristic comprising the steps of:
- 10 a. collecting equine sperm cells from a male species of an equine mammal;  
b. staining said equine sperm cells;  
c. establishing a cell source which supplies said equine sperm cells to be sorted;  
d. establishing a sheath fluid which is adapted to form droplets and which is compatible with said equine sperm cells;  
15 e. establishing a skim milk solution into which said equine sperm cells are collected;  
f. discriminating between said equine sperm cells according to a determination of their sex characteristic;  
g. entraining individual equine sperm cells in a droplet;  
20 h. sorting said droplets according to said sex of the individual equine sperm cells they contain; and  
i. collecting equine sperm cells having the desired sex characteristic in said skim milk solution.
- 46 A method of sorting equine sperm cells according to a determination of their sex  
25 characteristic as described in claim 45 wherein said step of establishing a skim milk solution into which said equine sperm cells are collected comprises the step of establishing a solution containing a skim milk extender as a collection fluid.

- 47      47      A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 46 wherein said step of establishing a skim milk solution into which said equine sperm cells are collected further comprises the step of establishing a solution containing about four percent egg yolk as a collection fluid.
- 5      48      A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 45 wherein said step of establishing a sheath fluid which is adapted to form droplets and which is compatible with said equine sperm cells comprises the step of establishing a sheath fluid containing a hepes buffered medium.
- 10      49      A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 45 wherein said step of sorting said droplets according to said sex of the individual equine sperm cells they contain comprises the step of sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry.
- 15      50      A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 49 wherein said step of sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry comprises the step of collecting live sperm of the desired sex at the rate of at least nine hundred live sperm per second.
- 20      51      A method of sorting equine sperm cells according to a determination of their sex characteristic as described in claim 49 wherein said step of sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry comprises the step of operating a high speed cell sorter at a pressure of at least about fifty pounds per square inch.
- 25      52      A method of practically producing an equine mammal involving sorted equine sperm cells wherein said sorted equine sperm cells are sorted according to a determination of

their sex characteristic through a method as described in claim 45 and further comprising the steps of:

- a. determining an estimated time of estrus of a female species of an equine mammal, said female species having two uterine horns, each uterine horns having a tip and a follicle;
- b. establishing an equine insemination sample containing at least some of said sorted equine sperm cells from said male species of said equine mammal and having a low number of said equine sperm cells relative to the typical artificial insemination dosage;
- c. non-surgically inserting at least a portion of said equine insemination sample in said female species of said equine mammal;
- d. artificially inseminating said female species of said equine mammal;
- e. fertilizing at least one equine egg within said female species of said equine mammal; and
- f. producing an equine offspring mammal of the desired sex.

53 A method of practically producing an equine mammal as described in claim 52 wherein said step of establishing an equine insemination sample containing at least some of said sorted equine sperm cells from said male species of said equine mammal and having a low number of said equine sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal and having a low number of said equine sperm cells relative to the typical artificial insemination dosage selected from the group consisting of: an equine insemination sample of no more than about five million sperm cells, and an equine insemination sample of no more than about twenty-five million sperm cells.

54 A method of practically producing an equine mammal as described in claim 53 and further comprising the step of ascertaining which uterine horn is ipsilateral to the preovulatory follicle and wherein said step of non-surgically inserting at least a portion of said equine insemination sample in said female species of said equine

mammal comprises the step of inserting at least a portion of said equine insemination sample near the tip of said uterine horn ipsilateral to the preovulatory follicle.

55 A method of practically producing an equine mammal as described in claim 53  
wherein said step of artificially inseminating said female species of said equine  
5 mammal comprises the step of artificially inseminating said female equine species on  
a single occasion close to ovulation.

56 A method of practically producing an equine mammal as described in claim 55  
wherein said step of artificially inseminating said female equine species on a single  
occasion close to ovulation comprises the step of comprises the step of artificially  
10 inseminating said female equine species both ipsi- and contra-lateral within the  
uterine horns of said equine mammal.

57 A method of practically producing an equine mammal as described in claim 54  
wherein said steps of inserting at least a portion of said equine insemination sample  
near the tip of said uterine horn ipsilateral to the preovulatory follicle, artificially  
15 inseminating said female species of said equine mammal, and fertilizing at least one  
equine egg within said female species of said equine mammal are each accomplished  
in a field environment.

58 A method of practically producing an equine mammal as described in claim 53  
wherein said step of establishing an equine insemination sample containing at least  
20 some of said equine sperm cells from said male species of said equine mammal  
comprises the step of establishing an equine insemination sample having a volume  
selected from the group: 0.2 ml, or 1ml.

59 An animal produced through use of a method as described in any of claims 45, 50, 53,  
57, or 58.

60 A method of flow cytometry accomplished through use of a method as described in any of claims 45, 48, or 50.

61 An equine-adapted flow cytometer system for isolating desired cells comprising:

- 5 a. an equine sperm cell source which supplies cells to be analyzed by the flow cytometer;
- b. a chemically coordinated sheath fluid source which creates a sheath fluid environment for said equine sperm cells;
- c. a nozzle through which said equine sperm cells pass while subjected to said sheath fluid environment;
- 10 d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
- e. a cell sensing system which responds to said equine sperm cells;
- f. a equine sperm cell sorter discrimination system which acts to sort said equine sperm cells having a desired sex characteristic; and
- 15 g. a skim milk solution collector into which said equine sperm cells having the desired sex characteristic are placed.

62 An equine-adapted flow cytometer system for isolating desired cells as described in claim 61 wherein said skim milk solution collector comprises a solution containing a skim milk semen extender.

20 63 An equine-adapted flow cytometer system for isolating desired cells as described in claim 62 wherein said skim milk solution collector further comprises a solution containing about four percent egg yolk.

25 64 An equine-adapted flow cytometer system for isolating desired cells as described in claim 62 wherein said chemically coordinated sheath fluid source comprises a sheath fluid containing a hepes buffered medium.



- 65 An equine-adapted flow cytometer system for isolating desired cells as described in claim 61 wherein said equine sperm cell sorter discrimination system comprises a high speed cell sorter.
- 5 66 An equine-adapted flow cytometer system for isolating desired cells as described in claim 65 wherein said a high speed cell sorter collects live sperm of the desired sex at the rate of at least nine hundred live sperm per second
- 67 An equine-adapted flow cytometer system for isolating desired cells as described in claim 65 wherein said a high speed cell sorter operates at a pressure of at least about fifty pounds per square inch.
- 10 68 A sexed sperm specimen produced through use of a system as described in any of claims 61, 62, 64, 65 or 66.
- 69 A sexed equine animal produced through use of a system as described in any of claims 61, 62, 64, 65 or 66.
- 70 A method of practically producing an equine mammal comprising the steps of:
- 15 a. determining an estimated time of estrus of a female species of an equine mammal, said female species having two uterine horns, each uterine horns having a tip and a follicle;
- b. collecting equine sperm cells from a male species of an equine mammal;
- c. determining the sex characteristic of a plurality of said equine sperm cells;
- 20 d. sorting said equine sperm cells according to the determination of their sex characteristic;
- e. establishing an equine insemination sample containing at least some of said sorted equine sperm cells from said male species of said equine mammal;
- f. non-surgically inserting at least a portion of said equine insemination sample in said female species of said equine mammal;
- 25 g. artificially inseminating said female species of said equine mammal;

- h. fertilizing at least one equine egg within said female species of said equine mammal; and
- i. producing an equine offspring mammal of the desired sex.

71 A method of practically producing an equine mammal as described in claim 70  
5 wherein said steps of determining the sex characteristic of a plurality of said equine sperm cells and sorting said equine sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. staining said equine sperm cells;
- b. sorting according to said sex of said equine sperm cells through the use of high  
10 speed flow cytometry; and
- c. concentrating said sorted equine sperm cells.

72 A method of practically producing an equine mammal as described in claim 71  
wherein said step of sorting according to said sex of said equine sperm cells through  
the use of high speed flow cytometry comprises the step of collecting live sperm of  
15 the desired sex at the rate of at least nine hundred live sperm per second.

73 A method of practically producing an equine mammal as described in claim 71  
wherein said step of sorting according to said sex of said equine sperm cells through  
the use of high speed flow cytometry comprises the step of operating a high speed cell  
sorter at a pressure of at least about fifty pounds per square inch.

20 74 A method of practically producing an equine mammal as described in claim 71  
wherein said step of sorting according to said sex of said equine sperm cells through  
the use of high speed flow cytometry comprises the step of collecting equine sperm  
cells having the desired sex characteristic in a skim milk solution.

75 A method of practically producing an equine mammal as described in claim 70  
25 wherein said step of establishing an equine insemination sample containing at least  
some of said equine sperm cells from said male species of said equine mammal

comprises the step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal and having a low number of said equine sperm cells relative to the typical artificial insemination dosage selected from the group consisting of: an equine insemination sample of no more than about five million sperm cells, and an equine insemination sample of no more than about twenty-five million sperm cells.

76 A method of practically producing an equine mammal as described in claim 72 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal and having a low number of said equine sperm cells relative to the typical artificial insemination dosage selected from the group consisting of: an equine insemination sample of no more than about five million sperm cells, and an equine insemination sample of no more than about twenty-five million sperm cells.

77 A method of practically producing an equine mammal as described in claim 75 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample having a volume selected from the group: 0.2 ml, or 1ml.

78 A method of practically producing an equine mammal as described in claim 76 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the step of establishing an equine insemination sample having a volume selected from the group: 0.2 ml, or 1ml.

79 A method of practically producing an equine mammal as described in claim 70 wherein said step of non-surgically inserting at least a portion of said equine

insemination sample in said female species of said equine mammal comprises the step of inserting at least a portion of said equine insemination sample deep within at least one of said uterine horns of said female species near the tip of said uterine horn.

80 A method of practically producing an equine mammal as described in claim 71  
5 wherein said step of non-surgically inserting at least a portion of said equine insemination sample in said female species of said equine mammal comprises the step of inserting at least a portion of said equine insemination sample deep within at least one of said uterine horns of said female species near the tip of said uterine horn.

81 A method of practically producing an equine mammal as described in claim 79  
10 wherein said step of artificially inseminating said female species of said equine mammal comprises the step of artificially inseminating said female equine species on a single occasion close to ovulation.

82 A method of practically producing an equine mammal as described in claim 80  
15 wherein said step of artificially inseminating said female species of said equine mammal comprises the step of artificially inseminating said female equine species on a single occasion close to ovulation.

83 A method of practically producing an equine mammal as described in claim 81 and  
20 further comprising the step of manipulating the ovulation of said female equine mammal prior to accomplishing the step of artificially inseminating said female species of said equine mammal.

84 A method of practically producing an equine mammal as described in claim 82 and  
further comprising the step of manipulating the ovulation of said female equine mammal prior to accomplishing the step of artificially inseminating said female species of said equine mammal.

- 85 A method of practically producing an equine mammal as described in claim 83  
wherein said step of manipulating the ovulation of said female equine mammal prior  
to accomplishing the step of artificially inseminating said female species of said  
equine mammal comprises the step of administering a gonadotropin releasing  
5 hormone to said female equine mammal.
- 86 A method of practically producing an equine mammal as described in claim 84 herein  
said step of manipulating the ovulation of said female equine mammal prior to  
accomplishing the step of artificially inseminating said female species of said equine  
mammal comprises the step of administering a gonadotropin releasing hormone to  
10 said female equine mammal.
- 87 A method of practically producing an equine mammal as described in claim 85 herein  
said step of artificially inseminating said female species of said equine mammal is  
accomplished at a time selected from the group consisting of: about thirty four hours  
after said step of administering said gonadotropin releasing hormone to said female  
15 equine mammal, about forty hours after said step of administering said gonadotropin  
releasing hormone to said female equine mammal, and between about thirty four  
hours to about forty hours after said step of administering said gonadotropin releasing  
hormone to said female equine mammal.
- 88 A method of practically producing an equine mammal as described in claim 86  
20 wherein said step of artificially inseminating said female species of said equine  
mammal is accomplished at a time selected from the group consisting of: about thirty  
four hours after said step of administering said gonadotropin releasing hormone to  
said female equine mammal, about forty hours after said step of administering said  
gonadotropin releasing hormone to said female equine mammal, and between about  
25 thirty four hours to about forty hours after said step of administering said  
gonadotropin releasing hormone to said female equine mammal.

89 A method of practically producing an equine mammal as described in claim 85 wherein said step of inserting at least a portion of said equine insemination sample deep within at least one of said uterine horns of said female species near the tip of said uterine horn comprises the steps of:

- 5 a. establishing a flexible probe having a sperm container;
- b. placing said flexible probe in the vagina of said female equine mammal;
- c. manipulating said flexible probe into said uterus of said female equine mammal;
- d. guiding said flexible probe into a uterine horn of said female equine mammal;
- 10 and
- e. gently manipulating said flexible probe per rectum as it is guided deep within said uterine horn of said female equine mammal to a location deep within said uterine horn of said female species near the tip of said uterine horn.

90 A method of practically producing an equine mammal as described in claim 86 wherein said step of inserting at least a portion of said equine insemination sample deep within at least one of said uterine horns of said female species near the tip of said uterine horn comprises the steps of:

- 15 a. establishing a flexible probe having a sperm container;
- b. placing said flexible probe in the vagina of said female equine mammal;
- 20 c. manipulating said flexible probe into said uterus of said female equine mammal;
- d. guiding said flexible probe into a uterine horn of said female equine mammal;
- and
- e. gently manipulating said flexible probe per rectum as it is guided deep within
- 25 said uterine horn of said female equine mammal to a location deep within said uterine horn of said female species near the tip of said uterine horn.

91 An animal produced through use of a method as described in any of claims 70, 72, 74, 77, 78, 79, or 89.

- 92 A method of practically producing an equine mammal comprising the steps of:
- a. determining an estimated time of estrus of a female species of an equine mammal, said female species having two uterine horns, each uterine horns having a tip and a follicle;
  - 5 b. collecting equine sperm cells from a male species of an equine mammal;
  - c. establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal and having a low number of said equine sperm cells relative to the typical artificial insemination dosage;
  - 10 d. non-surgically inserting at least a portion of said equine insemination sample in said female species of said equine mammal;
  - e. artificially inseminating said female species of said equine mammal;
  - f. fertilizing at least one equine egg within said female species of said equine mammal at success levels statistically comparable to the typical artificial insemination dosage; and
  - 15 g. producing an equine offspring mammal.

- 93 A method of practically producing an equine mammal as described in claim 92 and further comprising the step of ascertaining which uterine horn is ipsilateral to the preovulatory follicle and wherein said step of inserting at least a portion of said equine
- 20 insemination sample deep within at least one of said uterine horns of said female equine species comprises the step of inserting at least a portion of said equine insemination sample near the tip of said uterine horn ipsilateral to the preovulatory follicle.

- 94 A method of practically producing an equine mammal as described in claim 92
- 25 wherein said step of artificially inseminating said female species of said equine mammal comprises the step of artificially inseminating said female equine species on a single occasion close to ovulation.

95 A method of practically producing an equine mammal as described in claim 94  
wherein said step of artificially inseminating said female equine species on a single  
occasion close to ovulation comprises the step of comprises the step of artificially  
inseminating said female equine species both ipsi- and contra-lateral within the  
5 uterine horns of said equine mammal.

96 A method of practically producing an equine mammal as described in claim 92  
wherein said steps of inserting at least a portion of said equine insemination sample  
deep within at least one of said uterine horns of said female species near the tip of said  
uterine horn, artificially inseminating said female species of said equine mammal, and  
10 fertilizing at least one equine egg within said female species of said equine mammal  
are each accomplished in a field environment.

97 A method of practically producing an equine mammal as described in claim 92  
wherein said step of fertilizing at least one equine egg within said female species of  
said equine mammal at success levels statistically comparable to the typical artificial  
insemination dosage comprises the step of fertilizing at least one equine egg within  
15 said female species of said equine mammal at success levels selected from the group  
consisting of at least 90%, at least 81%, at least 75%, at least 65%, at least 60%, at  
least 57%, at least 40%, at least 35%, and at least 30%.

98 A method of practically producing an equine mammal as described in claim 92  
20 wherein said step of establishing an equine insemination sample having a low number  
of said equine sperm cells relative to the typical artificial insemination dosage  
comprises the step of establishing an equine insemination sample selected from the  
group consisting of: an equine insemination sample of no more than about forty  
million sperm cells, an equine insemination sample of no more than about fifty  
25 million sperm cells, and an equine insemination sample of no more than about one  
hundred million sperm cells.



- 99 A method of practically producing an equine mammal as described in claim 94  
wherein said step of establishing an equine insemination sample having a low number  
of said equine sperm cells relative to the typical artificial insemination dosage  
comprises the step of establishing an equine insemination sample selected from the  
5 group consisting of: an equine insemination sample of no more than about forty  
million sperm cells, an equine insemination sample of no more than about fifty  
million sperm cells, and an equine insemination sample of no more than about one  
hundred million sperm cells.
- 100 A method of practically producing an equine mammal as described in claim 97  
10 wherein said step of establishing an equine insemination sample having a low number  
of said equine sperm cells relative to the typical artificial insemination dosage  
comprises the step of establishing an equine insemination sample selected from the  
group consisting of: an equine insemination sample of no more than about forty  
million sperm cells, an equine insemination sample of no more than about fifty  
15 million sperm cells, and an equine insemination sample of no more than about one  
hundred million sperm cells.
- 101 A method of practically producing an equine mammal as described in claim 92  
wherein said step of establishing an equine insemination sample containing at least  
some of said equine sperm cells from said male species of said equine mammal  
20 comprises the steps of:  
a. determining the sex characteristic of a plurality of said equine sperm cells; and  
b. sorting said equine sperm cells according to the determination of their sex  
characteristic,  
and wherein said step of producing an equine offspring mammal comprises the step of  
25 producing an equine offspring mammal of the desired sex.
- 102 A method of practically producing an equine mammal as described in claim 94  
wherein said step of establishing an equine insemination sample containing at least

some of said equine sperm cells from said male species of said equine mammal comprises the steps of:

- a. determining the sex characteristic of a plurality of said equine sperm cells; and
- b. sorting said equine sperm cells according to the determination of their sex characteristic,

and wherein said step of producing an equine offspring mammal comprises the step of producing an equine offspring mammal of the desired sex.

103 A method of practically producing an equine mammal as described in claim 97 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the steps of:

- a. determining the sex characteristic of a plurality of said equine sperm cells; and
- b. sorting said equine sperm cells according to the determination of their sex characteristic,

and wherein said step of producing an equine offspring mammal comprises the step of producing an equine offspring mammal of the desired sex.

104 A method of practically producing an equine mammal as described in claim 102 wherein said steps of determining the sex characteristic of a plurality of said equine sperm cells and sorting said equine sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. staining said equine sperm cells;
- b. sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry; and
- c. concentrating said sorted equine sperm cells.

105 A method of practically producing an equine mammal as described in claim 104 wherein said step of sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry comprises the step of collecting live sperm of the desired sex at the rate of at least nine hundred live sperm per second.

- 106     A method of practically producing an equine mammal as described in claim 104 wherein said step of sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry comprises the step of operating a high speed cell sorter at a pressure of at least about fifty pounds per square inch.
- 5       107     A method of practically producing an equine mammal as described in claim 104 wherein said step of sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry comprises the step of collecting equine sperm cells having the desired sex characteristic in a skim milk solution.
- 108     An animal produced through use of a method as described in any of claims 92, 96, 97, 100, or 101.
- 109     A method of practically producing an equine mammal comprising the steps of:
- a.       determining an estimated time of estrus of a female species of an equine mammal, said female species having two uterine horns, each uterine horns having a tip and a follicle;
  - 15       b.       collecting equine sperm cells from a male species of an equine mammal;
  - c.       establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal and having a low number of said equine sperm cells relative to the typical artificial insemination dosage selected from the group consisting of: an equine  
20       insemination sample of no more than about five million sperm cells, and an equine insemination sample of no more than about twenty-five million sperm cells;
  - d.       non-surgically inserting at least a portion of said equine insemination sample in said female species of said equine mammal;
  - 25       e.       artificially inseminating said female species of said equine mammal;
  - f.       fertilizing at least one equine egg within said female species of said equine mammal; and
  - g.       producing an equine offspring mammal.

- 110 A method of practically producing an equine mammal as described in claim 109 and further comprising the step of ascertaining which uterine horn is ipsilateral to the preovulatory follicle and wherein said step of non-surgically inserting at least a portion of said equine insemination sample in said female species of said equine mammal comprises the step of inserting at least a portion of said equine insemination sample near the tip of said uterine horn ipsilateral to the preovulatory follicle.
- 5
- 111 A method of practically producing an equine mammal as described in claim 109 wherein said step of artificially inseminating said female species of said equine mammal comprises the step of artificially inseminating said female equine species on a single occasion close to ovulation.
- 10
- 112 A method of practically producing an equine mammal as described in claim 111 wherein said step of artificially inseminating said female equine species on a single occasion close to ovulation comprises the step of artificially inseminating said female equine species both ipsi- and contra-lateral within the uterine horns of said equine mammal.
- 15
- 113 A method of practically producing an equine mammal as described in claim 109 wherein said steps of non-surgically inserting at least a portion of said equine insemination sample in said female species of said equine mammal, artificially inseminating said female species of said equine mammal, and fertilizing at least one equine egg within said female species of said equine mammal are each accomplished in a field environment.
- 20
- 114 A method of practically producing an equine mammal as described in claim 109 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the steps of:
- 25
- a. determining the sex characteristic of a plurality of said equine sperm cells; and

b. sorting said equine sperm cells according to the determination of their sex characteristic,  
and wherein said step of producing an equine offspring mammal comprises the step of producing an equine offspring mammal of the desired sex.

5      115    A method of practically producing an equine mammal as described in claim 111 wherein said step of establishing an equine insemination sample containing at least some of said equine sperm cells from said male species of said equine mammal comprises the steps of:

10      a.      determining the sex characteristic of a plurality of said equine sperm cells; and  
b.      sorting said equine sperm cells according to the determination of their sex characteristic,  
and wherein said step of producing an equine offspring mammal comprises the step of producing an equine offspring mammal of the desired sex.

15      116    A method of practically producing an equine mammal as described in claim 115 wherein said steps of determining the sex characteristic of a plurality of said equine sperm cells and sorting said equine sperm cells according to the determination of their sex characteristic comprise the steps of:

20      a.      staining said equine sperm cells;  
b.      sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry; and  
c.      concentrating said sorted equine sperm cells.

25      117    A method of practically producing an equine mammal as described in claim 116 wherein said step of sorting according to said sex of said equine sperm cells through the use of high speed flow cytometry comprises the step of collecting live sperm of the desired sex at the rate of at least nine hundred live sperm per second.

118    A method of practically producing an equine mammal as described in claim 116 wherein said step of sorting according to said sex of said equine sperm cells through

the use of high speed flow cytometry comprises the step of operating a high speed cell sorter at a pressure of at least about fifty pounds per square inch.

- 119 A method of practically producing an equine mammal as described in claim 116  
wherein said step of sorting according to said sex of said equine sperm cells through  
5 the use of high speed flow cytometry comprises the step of collecting equine sperm  
cells having the desired sex characteristic in a skim milk solution.
- 120 A method of practically producing an equine mammal as described in claim 109 and  
further comprising the step of administering an equine pituitary extract to said to said  
female equine mammal to enhance the probability at which said step of fertilizing at  
10 least one equine egg within said female species of said equine mammal occurs.
- 121 A method of practically producing an equine mammal as described in claim 109  
wherein said step of establishing an equine insemination sample containing at least  
some of said equine sperm cells from said male species of said equine mammal  
comprises the step of establishing an equine insemination sample having a volume  
15 selected from the group: 0.2 ml, or 1ml.
- 122 A method of practically producing an equine mammal as described in claim 110  
wherein said step of establishing an equine insemination sample containing at least  
some of said equine sperm cells from said male species of said equine mammal  
comprises the step of establishing an equine insemination sample having a volume  
20 selected from the group: 0.2 ml, or 1ml.
- 123 A method of practically producing an equine mammal as described in claim 92  
wherein said step of non-surgically inserting at least a portion of said equine  
insemination sample in said female species of said equine mammal comprises the step  
of inserting at least a portion of said equine insemination sample deep within at least  
25 one of said uterine horns of said female species near the tip of said uterine horn.

- 124 A method of practically producing an equine mammal as described in claim 109  
wherein said step of non-surgically inserting at least a portion of said equine  
insemination sample in said female species of said equine mammal comprises the step  
of inserting at least a portion of said equine insemination sample deep within at least  
5 one of said uterine horns of said female species near the tip of said uterine horn.
- 125 A method of practically producing an equine mammal as described in claim 111  
wherein said step of non-surgically inserting at least a portion of said equine  
insemination sample in said female species of said equine mammal comprises the step  
of inserting at least a portion of said equine insemination sample deep within at least  
10 one of said uterine horns of said female species near the tip of said uterine horn.
- 126 A method of practically producing an equine mammal as described in claim 110  
wherein said step of non-surgically inserting at least a portion of said equine  
insemination sample in said female species of said equine mammal comprises the step  
of inserting at least a portion of said equine insemination sample deep within at least  
15 one of said uterine horns of said female species near the tip of said uterine horn.
- 127 A method of practically producing an equine mammal as described in claim 125 and  
further comprising the step of manipulating the ovulation of said female equine  
mammal prior to accomplishing the step of artificially inseminating said female  
species of said equine mammal.
- 20 128 A method of practically producing an equine mammal as described in claim 126 and  
further comprising the step of manipulating the ovulation of said female equine  
mammal prior to accomplishing the step of artificially inseminating said female  
species of said equine mammal.
- 129 A method of practically producing an equine mammal as described in claim 127  
wherein said step of manipulating the ovulation of said female equine mammal prior  
25 to accomplishing the step of artificially inseminating said female species of said

equine mammal comprises the step of administering a gonadotropin releasing hormone to said female equine mammal.

130 A method of practically producing an equine mammal as described in claim 128 wherein said step of manipulating the ovulation of said female equine mammal prior to accomplishing the step of artificially inseminating said female species of said equine mammal comprises the step of administering a gonadotropin releasing hormone to said female equine mammal.

131 A method of practically producing an equine mammal as described in claim 129 wherein said step of artificially inseminating said female species of said equine mammal is accomplished at a time selected from the group consisting of: about thirty four hours after said step of administering said gonadotropin releasing hormone to said female equine mammal, about forty hours after said step of administering said gonadotropin releasing hormone to said female equine mammal, and between about thirty four hours to about forty hours after said step of administering said gonadotropin releasing hormone to said female equine mammal.

132 A method of practically producing an equine mammal as described in claim 130 wherein said step of artificially inseminating said female species of said equine mammal is accomplished at a time selected from the group consisting of: about thirty four hours after said step of administering said gonadotropin releasing hormone to said female equine mammal, about forty hours after said step of administering said gonadotropin releasing hormone to said female equine mammal, and between about thirty four hours to about forty hours after said step of administering said gonadotropin releasing hormone to said female equine mammal.

133 A method of practically producing an equine mammal as described in claim 129 wherein said step of inserting at least a portion of said equine insemination sample deep within at least one of said uterine horns of said female species near the tip of said uterine horn comprises the steps of:



- a. establishing a flexible probe having a sperm container;
- b. placing said flexible probe in the vagina of said female equine mammal;
- c. manipulating said flexible probe into said uterus of said female equine mammal;
- 5 d. slowly guiding said flexible probe into a uterine horn of said female equine mammal; and
- e. gently manipulating said flexible probe per rectum as it is guided deep within said uterine horn of said female equine mammal to a location deep within said uterine horn of said female species near the tip of said uterine horn.

10 134 hod of practically producing an equine mammal as described in claim 130 wherein said step of inserting at least a portion of said equine insemination sample deep within at least one of said uterine horns of said female species near the tip of said uterine horn comprises the steps of:

- a. establishing a flexible probe having a sperm container;
- 15 b. placing said flexible probe in the vagina of said female equine mammal;
- c. manipulating said flexible probe into said uterus of said female equine mammal;
- d. slowly guiding said flexible probe into a uterine horn of said female equine mammal; and
- 20 e. gently manipulating said flexible probe per rectum as it is guided deep within said uterine horn of said female equine mammal to a location deep within said uterine horn of said female species near the tip of said uterine horn.

135 An animal produced through use of a method as described in any of claims 109, 113, 114, 117, 119, 120, 121, 122, 123, 127, or 133 .